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(57) Abstract

The invention relates to a lyophilized composition which comprises a granulocyte macrophage colony stimulating factor (GM-CSF), a pharmaceutically acceptable bulking agent, a polyoxyethylene sorbitan fatty acid ester and a basic amino acid. The compositions according to the present invention are useful in a method of treatment of the human or animal body, e.g. in the treatment of neutropenic disorders of cancer patients after chemotherapy.

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Lyophilized stable pharmaceutical compositions containing a granulocyte macrophage colony stimulating factor.

The present invention relates to freeze-dried (lyophilized) compositions containing granulocyte-macrophage colony stimulating factor (GM-CSF).

GM-CSF is a glycoprotein able to control the proliferation, maturation and differentiation of myeloid progenitor cells to form differentiated granulocytes, macrophages and certain related hemopoietic cells.

GM-CSF also enhances the function of mature blood cells and stimulates the production of other cytokines such as, for example, interleukin 1 and M-CSF.

It is known that it is very difficult to prepare stable pharmaceutical compositions containing proteins since these substances easily undergo processes of degradation with consequent decrease or loss of their pharmacological activity.

Degradation pathways for proteins can be separated into two distinct classes, involving both chemical and physical instability.

First, chemical instability can include proteolysis, deamidation, oxidation, racemization and ß-elimination. Physical instability refers to processes such as aggregation, precipitation, denaturation and adsorption to surface.

Temperature, light, and humidity are the most important factors responsible for the above mentioned drop in the activity of the proteins.

These molecules are also at risk of microbial degradation due to adventitious contaminations of the solution during purification or storage.

Freeze-drying (also known as lyophilization) is a process commonly used in the manufacture of protein products that are

insufficiently stable for distribution and use in aqueous solution, even if frozen.

In general, pharmaceutical protein products are not pure proteins, but are formulated products in which general chemical components have been added for specific purposes, e.g. to improve stability during the freeze-drying process and/or during subsequent storage. It would therefore be desirable to prepare a lyophilized composition containing GM-CSF with a long shelf life, able to endure physico-chemical and microbial degradations.

According to the present invention there is provided a lyophilized composition which comprises a granulocyte macrophage colony stimulating factor (GM-CSF), a pharmaceutically acceptable bulking agent, a polyoxyethylene sorbitan fatty acid ester and a basic amino acid. Optionally said lyophilized compositions may also contain a suitable buffering agent such as, e.g. a monobasic alkali metal phosphate, preferably monobasic sodium phosphate.

The GM-CSF contained in the pharmaceutical preparations of the present invention may be any GM-CSF molecule though it is, preferably, a recombinantly prepared GM-CSF, as obtained, for example, by expressing a recombinant DNA in an appropriate microbial host cell such as, e.g., a bacterial host, e.g. E. coli, a yeast or a mammalian cell. The GM-CSF is preferably human GM-GSF. Among the GM-CSFs a preferred one for use in the invention is the human GM-CSF whose amino acid sequence is shown in

SEQ ID NO:1. This GM-CSF is a preferred recombinant GM-CSF.
The term GM-CSF, according to the invention, includes also muteins obtained by deletions, insertions or substitutions of aminoacid residues as well as extensions by way of aminoacid residues..
A deletion, insertion, substitution or extension may be N-terminal, C-terminal or internal to the basic sequence and may comprise one or more amino acids.
A further preferred embodiment of the present invention is the mutein Leu^{23} GM-CSF, i.e. a mutein of human GM-CSF wherein the amino acid naturally present in the position 23 of the human GM-CSF sequence shown in SEQ NO:1 is substituted by leucyn.

In the compositions of the invention GM-CSF may be present in a very small amount. For example a pharmaceutical composition containing from 0.1 to 5 mg of GM-CSF, preferably from 250 µg to 750 µg of GM-CSF, may be administered. The amount of GM-CSF in the composition of the present invention is preferably from 0.1 to 5%, most preferably from 0.1 to 1%, by weight of the bulking agent.

A pharmaceutically acceptable bulking agent may be any bulking agent suitable for use in freeze-drying such as, for example, mannitol, lactose, polyvinylpyrrolidone (PVP), dextran or glycine; of these, mannitol is preferred.

Examples of polyoxyethylene sorbitan fatty acid esters include partial C_{12-20} saturated or unsaturated fatty acid esters of sorbitol and its mono- and di-anhydrides copolymerised with ethylene oxide. Typically, from 10 to 40, for example about 20 moles of ethylene oxide for each mole of sorbitol and its anhydrides will be present. Polyoxyethlene sorbitan fatty acid esters are known generally as polysorbates.

Examples of polysorbates include polysorbate 20 (polyoxyethylene 20 sorbitan monolaurate, Chemical Abstracts CAS Reference No. 9005-64-5) which is a mixture of partial lauric esters or sorbitol and its mono- and di-anhydrides copolymerized with approximately 20 moles of ethylene oxide for each mole of sorbitol and its anhydrides, polysorbate 40 (polyoxyethylene 20 sorbitan monopalmitate, CAS No. 9005-66-7), polysorbate 60 (polyoxyethylene 20 sorbitan monostearate CAS No. 9005-67-8), polysorbate 65 (polyoxyethylene 20 sorbitan tristearate, CAS No. 9005-71-4), polysorbate 80 (polyoxyethylene 20 sorbitan monoleate, CAS No. 9004-65-6) and polysorbate 85 (polyoxyethylene 20 sorbitan trioleate, CAS No. 9005-70-3). Of these the preferred polysorbate is polysorbate 80, also known as Tween 80. In the compositions of the invention the amount of polysorbate is generally from 0.01% to 25%, preferably from 0.1% to 1%, by weight of the bulking agent.

Typical examples of basic amino acids for use in making the stable GM-CSF-containing pharmaceutical preparations of the present invention include lysine and arginine. These may be used either singly or in admixture. The amino acids are preferably used in an amount ranging from 0.001% to 5%, most preferably from 0.1% to 2%, by weight of the bulking agent.

As already said, if desired, the solution may also be buffered, e.g. to a pH of about 6.5, with a pharmaceutically acceptable buffering agent, such as monobasic sodium phosphate.

Compositions of the present invention will normally be formulated in solution prior to freeze-drying. The solution may be freeze-dried in any quantity although preferably, the solution will be divided into aliquots containing from 10 to 1000, for example from 100 to 500, most preferably 250, µg of GM-CSF.

These aliquots will be freeze-dried separately, e.g. in individual glass vials. Before the solution is freeze-dried, it may be sterilized by filtration.

For example, a 0.22 µm polyvinylidenedifluoride membrane filter may be used for this purpose, to prevent adsorption of the molecules on the surface.

Using HPLC analysis carried out before and after such filtration, we have found that GM-CSF is consistently recovered on a quantitative basis.

A typical freeze-drying cycle used for GM-CSF containing pharmaceutical preparation may be, e.g., as follows:

(a) freeze at -45°C, and maintain this temperature for four hours;

(b) primary drying at -45°C to +10°C for approximately twenty hours, with vacuum level less than 13.3 Pa (0.1 torr) and a condenser temperature of -60°C; and

(c) secondary drying at 10°C to +25°C for approximately twenty-four hours, with the same vacuum and condenser temperature as described in (b) above.

Variations of this protocol which do not substantially alter the stability of the GM-CSF may be made.

Aliquots of the composition of the present invention may be dispensed into sterile vials. Sterile glass vials can be suitable.

It is clearly known that proteins adhere to glass surface, and we have found that, when the freeze-dried product of the present invention is reconstituted in a glass vial, some loss of protein, possibly due to adhesion on the glass surface, occurs.

However, we have found that coating the glass vials with silicon, in order to minimise sticking, successfully overcomes this problem.

The glass vials can be sealed with conventional rubber stoppers (chloro butyl rubber) because no losses of protein, due to adsorption of GM-CSF to the rubber surface, was observed. The lyophilized composition of the present invention may be stored for example under an inert gas, e.g. nitrogen.

The freeze-dried product composition of the invention may be reconstituted using any aqueous physiologically acceptable sterile solvent. Preferably, the solvent used will provide a reconstituted solution with a pH between about 5 and about 7.0, most preferably about 6.5 Preferably, a 0.9% aqueous solution of sodium chloride (i.e. physiological saline) is used as the reconstitution solvent.

Optionally, the solution may contain an effective amount of an anti-microbial preservative agent such as, for example, benzalkonium chloride, in order to inhibit the microbial activity in reconstituted solutions of the present invention.

The invention thus provides both a method for preparing a lyophilized GM-CSF composition according to the invention, which process comprises mixing, in aqueous solution, GM-CSF, a pharmaceutically acceptable bulking agent, a polyoxyethylene sorbitan fatty acid ester and a basic amino acid, and freeze-drying the resulting solution;

and a method of preparing an aqueous injectable GM-CSF solution which comprises reconstituting the freeze-dried composition of the present invention with a physiologically acceptable sterile aqueous solvent.

The present invention also provides a kit containing the lyophilized compositions described above in a sterile vial and a physiologically acceptable sterile aqueous solvent for reconstitution of the lyophilized composition.

The compositions or kits according to the present invention are useful in a method of treatment of the human or animal body, e.g. in the treatment of neutropenic disorders of cancer patients after chemotherapy.

The Examples which follow illustrate aspects of the present invention without limiting its scope.

In the following Examples, the GM-CSF is a recombinantly prepared human GM-CSF having the sequence shown in SEQ ID NO:1, which is prepared following the conventional recombinant techniques well known in the art (DNA 6(3), 221-229, 1987, Current Microbiology 17, 321-332, 1988). Similar techniques may be followed for preparing any GM-CSF molecule according to the invention.

This compound will be referred to in the present specification as rh GM-CSF.

It is, e.g., obtained as a solid bulk at a concentration of active substance of approximately 880 µg/mg expressed as protein content measured by the biuret reaction. This solid bulk is stored at about -20°C. It was observed that thawing and diluting this bulk to a

concentration of about 125 µg/ml using a 2.5% mannitol solution does not affect protein stability. HPLC analysis of this solution shows that the active drug substance (rh GM-CSF) is quantitatively recovered.

At first, studies were conducted to choose the best bulking agent suitable for the formulation.

EXAMPLE 1

Solutions containing rh GM-CSF (125 µg/ml), mannitol, lactose, polyvinylpyrrolidone (PVP), were prepared aseptically, filled into vials (nominal volume 2.0 ml) and freeze-dried. The appearance of the reconstituted solution and the effect of storage on the protein potency in the final freeze-dried formulation, were checked through accelerated stability studies (25°-35°C).

The results, as summarized in Table 1, demonstrate that, among the test substances, the most suitable bulking agent for the pharmaceutical compositions of the present invention is mannitol.

TABLE 1

COMPOSITION	AMOUNT	RESIDUAL rh GM-CSF% (HPLC) AFTER: 7 days 25°	7 days 35°	APPEARANCE AFTER RECONSTITUTION
Mannitol	50 mg	96.5	91	clear and clean colourless solution free from visible particles of foreign matter
Lactose	50 mg	98.9	92.3	slightly opalescent solution; same particulate matter in suspension
PVP	40 mg	91.3	85.5	slightly opalescent solution; same particulate matter in suspension

Example 2

According to the literature (P.P. De Luca and M.W. Townsend J. Par. Sci. and Tecn. Vol. 42 No. 6, pag. 190), a lyoprotectant is defined as a compound that stabilizes and prevents the degradation of the proteins both during freeze-drying and afterwards, during storage, whereas a cryoprotectant only infers protection from freezing damage.

Based on these theoretical considerations, experimental work was thus undertaken to determine the protective capacity on GM-CSF of a number of compounds which might act as lyoprotectants.

Polysorbate 80 (Tween 80), sodium carboxymethyl cellulose (NaCMC), sodium chloride, arginine (Arg), lysine (Lys), aspartic acid (Asp) and meglumine were tested as possible protective agents. Solutions containing rh GM-CSF (125 µg/ml), mannitol (50 mg/ml) as bulking agent, monobasic sodium phosphate (pH 6.5) as buffering agent and a suitable concentration of each potential stabilizer, were prepared aseptically, filled into vials (nominal volume 2.0 ml) and then freeze-dried.

The effect of storage on the protection potency was checked through accelerated stability studies (35°C).

Basic experimental results are summarized in Table 2.

The freeze-dried formulation containing only mannitol as bulking agent underwent about 10% potency loss after one w ek of storage.

The presence of lyoprotectants such as Arginine significantly improved protein stability.

Other tested compounds such as Lysine, Aspartic Acid, meglumine proved to be ineffective as stabilizers. Data are presented in Table 2 only for Asp, but also the other tested compounds behaved similarly.

Polysorbate 80 proved to be ineffective if used alone, but surprisingly this stabilizer worked well in combination with arginine.

The low stabilizing activity of polysorbate 80 might be expected, due to the low coordination power of this additive towards the water molecules. On the contrary, the synergistic effect of polysorbate 80 with arginine was quite unexpected.

TABLE 2

Recombinant human GM-CSF (rh GM-CSF) preformulation studies. Accelerated stability results of different freeze-dried formulations, containing GM-CSF (250 mcg/ml), Mannitol (50 mg/ml) and Monobasic Sodium Phosphate (pH 6.5).

COMPOSITION mg			RESIDUAL rh GM-CSF % (HPLC assay) after		
Asp	Arg	Tween 80	1 week 35°C	2 weeks 35°C	3 weeks 35°C
1	-	-	86.8	n.d.	72.0
-	1	-	98.4	97.8	92.3
-	-	0.1	n.d.	86.3	n.d.
-	1	0.05	n.d.	96.1	n.d.

In Table 2 n.d. means not determined.

Example 3

Composition of rh GM-CSF formulation stabilized with polysorbate 80 and Arginine.

	per vial ***	per 2000 vials
rh GM-CSF	0.2875 mg*	575 mg*
Mannitol	52.5000 mg	105 g
Polysorbate 80	0.0525 mg	105 mg
L-Arginine	1.05 mg	1.1 g
Monobasic Sodium Phosphate	2.898 mg	5.8 g
Sodium hydroxide qs to	6.5 pH	qs to pH 6.5
Water for Injection ** qs to	2.0 ml	qs to 4.0 l

* Including 10% overage to compensate for losses during manufacture

** During freeze-drying water for injections is removed

*** a 5% overfill of the rh GM-CSF/Mannitol/Polysorbate 80/L-Arginine/Monobasic Sodium Phosphate solution is included.

The formulation was freeze-dried and individual vials were sealed under nitrogen.

EXAMPLE 4

Stability of compositions of the invention

Freeze-dried vials containing compositions according to the present invention comprising about 250 g of GM-CSF were examined for long term stability over various periods of time at different temperatures. The following parameters were examined and the acceptable standards are also given:

- Appearance:	colourless glass vials, containing a compact, white, freeze-dried cake or mass, determined by visual inspection
- Identification:	Same retention time as rh GM-CSF working standard (HPLC method as illustrated below)
- RP-HPLC assay:	85-115% of the label chain
- Water:	not more than 3%
- Appearance after reconstitution*:	clear and clean colourless solution, essentially free from visible particles of foreign matter
- pH after reconstitution*:	6-7

* The contents of the vials are dissolved in 2 ml of the required solvent (0.9% Sodium Chloride Injection, BP).

The HPLC methodology employed is as follows:

Materials

GM-CSF, working standard

Acetonitrile, HPLC grade

Water, HPLC grade

Trifluoroacetic acid, analytical grade

Phosphate buffer at pH 7.5 : Transfer 7.3 g of sodium chloride and 3.2 g of Sodium dihydrogen phosphate in a 1000 ml volumetric flask.

Dissolve with about 800 ml of distilled water and bring the pH to 7.5 with 2N sodium hydroxide.

Fill to the mark with distilled water.

Equipment

Liquid chromatograph Milton Roy model CM 4000, or equivalent, equipped with:

- chromatographic column : (length 150 mm, internal diameter 4.6 mm) filled with PLRP-S 300 A (average particle size 8 µm), supplied by Polymer Laboratories Ltd, Shropshire, U.K. or equivalent
- injection valve: Rheodyne model 7125, or equivalent, fitted with a 100 µl sample loop
- detector: Shimadzu model SPD 6A, or equivalent
- integrating recorder: SP 4270 (Spectra-Physics), or equivalent

Membrane filter, 0.22 µm porosity, Millipore Durapore GVWP, or equivalent

High precision laboratory glassware

Solutions

Mobile phase (A) consisting of water, containing

0.1% of triflu roacetic acid (w/v), filtered through the membrane filter and deaerated.

Mobile phase (B) consisting of 95% acetonitrile-5% water containing 0.1% of trifluoroacetic acid (w/v), filtered through the membrane filter and deaerated.

Standard solution

Dissolve about 6 mg, exactly weighed, of GM-CSF in 50 ml of phosphate buffer at pH 7.5.

The standard solution must be freshly prepared and used within a working day.

Sample solution

Prepare the sample solution using at least five freeze-dried vials.

The content of each vial dosed at 250 µg of GM-CSF is dissolved in 2.0 ml of phosphate buffer at pH 7.5, then a pool is made with all prepared solutions.

Chromatographic (HPLC) conditions

The standard and sample solution are alternatively injected at least 2 times into the liquid chromatograph under the following experimental conditions:

Column temperature	: room temperature (22 ± 2°C)
Mobile phase flow-rate	: 1 ml/min
Analytical wavelength	: 215 ± 1 nm

Gradient conditions : time (min) A% B%

time (min)	A%	B%
0	82	18
15	58	42
21	53	47
23	53	47
33	82	18

Detector sensitivity : the detector "computer" output is connected to integrator for maximum sensitivity

Injection volume : 100 µl

Integrating recorder attenuation : 1024

Chart speed : 0.5 cm/min

The results obtained from studies of accelerated stability, for the formulation illustrated in Example 3 are reported in the following Tables 3 to 8 with reference to two different batches.

TABLE 3 - Accelerated stability data of rh GM-CSF freeze-dried - Batch No. TF/23765

Active drug substance Batch No.: OP52

COMPOSITION of Example 3

Tests	Initial control	35°C 2 weeks	4 weeks	2 mos
Appearance	Complies	Unchanged		
RP-HPLC assay				
. mcg/vial	256	252.2	249.1	243.9
. % initial	100	98.5	97.3	95.3
Water %	1.04	n.d.	n.d.	n.d.
Appearance (reconstituted solution)	Complies	Unchanged		
pH (reconstituted solution)	6.88	6.83	6.84	6.89

TABLE 4 - Long term stability data of rh GM-CSF freeze-dried vials - Batch No. TF/23765
Active drug substance Batch No: OP.52

COMPOSITION of Example 3

Tests	Initial control	25°C 4 weeks	2 mos	3 mos	6 mos
Appearance	Complies	Unchanged			
RP-HPLC assay					
. mcg/vial	256	251.4	249.3	252.12	243.3
. % initial	100	98.2	97.4	98.5	95.06
Water %	1.04	n.d.	n.d.	n.d.	
Appearance (reconstituted solution)	Complies	Unchanged			
pH (reconstituted solution)	6.88	6.87	6,87	6.89	6.8

TABLE 4 - Long term stability data of rh GM-CSF freeze-dried vials - Batch No. TF/23765
Active drug substance Batch No: OP.52

COMPOSITION of Example 3

Tests	Initial control	4°C				
		4 weeks	2 mos	3 mos	6 mos	9 mos
Appearance	Complies	Unchanged				
RP-HPLC assay						
. mcg/vial	256	256.5	255.46	263.3	256.63	251.0
. % initial	100	100.2	99.8	102.9	100.27	98.1
Water %	1.04	n.d.	n.d.	n.d.	n.d.	n.d.
Appearance (reconstituted solution)	Complies	Unchanged				
pH (reconstituted solution)	6.88	6.84	6.88	6.86	6.9	6.9

TABLE 6 - Accelerated stability data of rh GM-CSF freeze-dried vials - Batch No. NP8730/29F
Active drug substance Batch No: OP44/A

COMPOSITION of Example 3

Tests	Initial control	35°C		
		2 weeks	4 weeks	2 mos.
Appearance	Complies	Unchanged		
RP-HPLC assay				
. mcg/vial	258	247.9	243.5	227.5
. % initial	100	96.1	94.4	91.0
Water %	1.5	n.d.	n.d.	n.d.
Appearance (reconstituted solution)	Complies	Unchanged		
pH (reconstituted solution)	6.78	6.74	6.74	6.73

TABLE 7 - Long term stability data of rh GM-CSF freeze-dried vials - Batch No. NP8730/29F
Active drug substance Batch No:OP44/A

COMPOSITION of Example 3

Tests	Initial control	25°C			
		2 weeks	4 weeks	2 mos	6 mos
Appearance	Complies	Unchanged			
RP-HPLC assay					
. mcg/vial	258		247.9	245.8	236.09
. % initial	100		96.1	95.3	91.5
Water %	1.5		n.d.	n.d.	n.d.
Appearance (reconstituted solution)	Complies	Unchanged			
pH (reconstituted solution)	6.78		6.74	6.74	6.76

TABLE 4 - Long term stability data of rh GM-CSF freeze-dried vials - Batch No. NP 8730/29F
Active drug substance Batch No: OP 44/A

COMPOSITION of Example 3

Tests	Initial control	4°C					
		4 weeks	2 mos	3 mos	6 mos	9 mos	12 mos
Appearance	Complies	Unchanged					
RP-HPLC assay							
. mcg/vial	258	259.03	251.3	n.d.	250.42	n.d.	245.88
. % initial	100	100.4	97.4	n.d.	97.06	n.d.	95.3
Water %		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Appearance (reconstituted solution)	Complies	Unchanged					
pH (reconstituted solution)	6.78	6.72	6.78	6.71	6.78	n.d.	6.73

SEQUENCE LISTING

(1) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 127 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala Pro Ala Arg Ser Pro Ser Pro Ser Thr Gln Pro Trp Glu His Val
1 5 10 15

Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser Arg Asp Thr
20 25 30

Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu Met Phe Asp
35 40 45

Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys Gln
50 55 60

Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu Thr Met Met
65 70 75 80

Ala Ser His Tyr Lys Gln His Cys Pro Pro Thr Pro Glu Thr Ser Cys
85 90 95

Ala Thr Gln Thr Ile Thr Phe Glu Ser Phe Lys Glu Asn Leu Lys Asp
100 105 110

Phe Leu Leu Val Ile Pro Phe Asp Cys Trp Glu Pro Val Gln Glu
115 120 125

CLAIMS

1. A lyophilized composition which comprises a granulocyte macrophage colony stimulating factor (GM-CSF), a pharmaceutically acceptable bulking agent, a polyoxyethylene sorbitan fatty acid ester and a basic amino acid.

2. A composition according to claim 1 which additionally comprises a buffering agent.

3. A composition according to claim 2 in which the buffering agent is monobasic sodium phosphate.

4. A composition according to anyone of the preceding claims in which the bulking agent is mannitol.

5. A composition according to anyone of the preceding claims in which the polyoxyethylene sorbitan fatty acid ester is polysorbate 80.

6. A composition according to anyone of the preceding claims in which the basic amino acid is arginine.

7. A composition according to anyone of the preceding claims in which the GM-CSF is a recombinant human GM-CSF having the amino acid sequence shown in SEQ ID NO:1.

8. A composition according to claims 1-6 in which the GM-CSF is the mutein Leu^{23} GM-CSF.

9. A composition according to anyone of the preceding claims in a sealed sterile glass vial.

10. Method for preparing a lyophilized composition according to claim 1 which comprises mixing, in aqueous solution, GM-CSF, a pharmaceutically acceptable bulking agent a polyoxyethylene sorbitan fatty acid ester and a basic amino acid, and freeze-drying the resulting solution.

11. A method for preparing an aqueous GM-CSF solution which comprises reconstituting a freeze-dried composition according to anyone of claims 1 to 8 with a physiologically acceptable aqueous sterile solvent.

12. A kit comprising
a) a composition according to anyone of claims 1 to 8 and
b) a physiologically acceptable aqueous sterile solvent for reconstituting said composition.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/02084

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02; A61K47/18; A61K47/26; A61K9/14

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K ; C12P ; C12N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	WO,A,9 008 554 (SCHERING CORPORATION) 9 August 1990 see page 7, line 12 - line 27 ---	1-12
A	WO,A,8 910 407 (GENETICS INSTITUTE, INC.) 2 November 1989 see the whole document ---	1-2,5, 10-12
A	EP,A,0 355 811 (CHUGAI SEIYAKU KABUSHIKI KAISHA) 28 February 1990 see examples 3,4 ---	1-5,7-12
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° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
03 DECEMBER 1992	15. 12. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	LE CORNEC N.D.R.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9202084 SA 64273

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 03/12/92
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